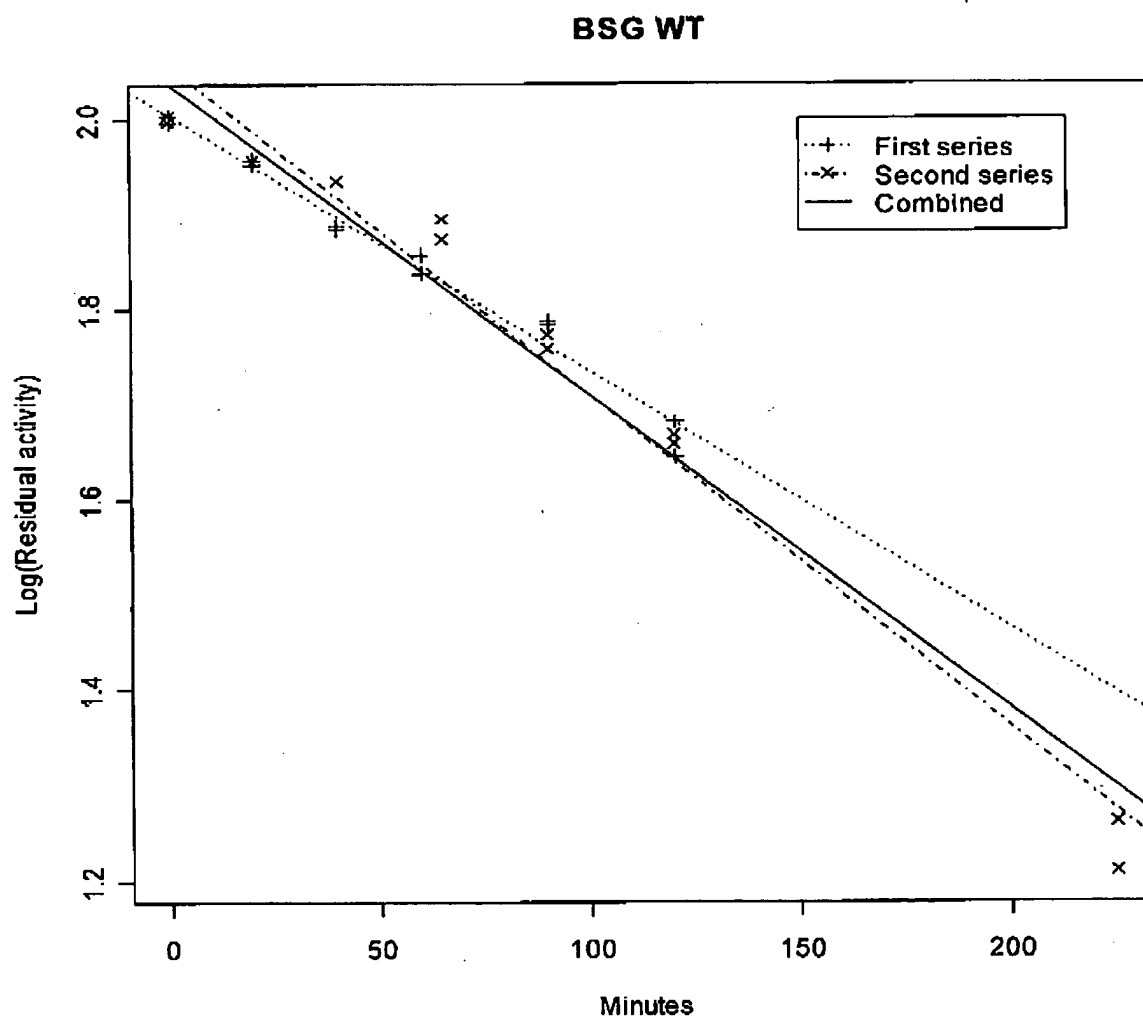


Appendix 1:***Differences between data-series, BSG:***

BSG-WT



Summary of statistical analysis.

Below is a screen-dump from the statistical analysis, showing that there is a significant difference in the slope in the two data series. The p-value for same slope is underlined.

The analysis was done in R version 1.8.1 (<http://www.r-project.org>). The data for the BSG WT is held in the data-frame `bsg.wt` as shown in the table above. In the data-frame the time is called `var1`, the residual activity is `var2` and `var3` is a factor over the two series of experiments. The output shows the effect of the factor on a linear regression on the Log(residual activity) over incubation time.

```
> summary(lm(log10(bsg.wt$var2)~bsg.wt$var1*bsg.wt$var3))
```

Call:

```
lm(formula = log10(bsg.wt$var2) ~ bsg.wt$var1 + bsg.wt$var3)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-0.063233	-0.012558	0.001456	0.020149	0.063980

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.0043563	0.0165332	121.233	< 2e-16 ***
bsg.wt\$var1	-0.0027017	0.0002416	-11.183	4.68e-10 ***
bsg.wt\$var32	0.0520051	0.0226679	2.294	0.0327 *
bsg.wt\$var1:bsg.wt\$var32	-0.0007776	0.0002771	-2.806	<u>0.0109</u> *

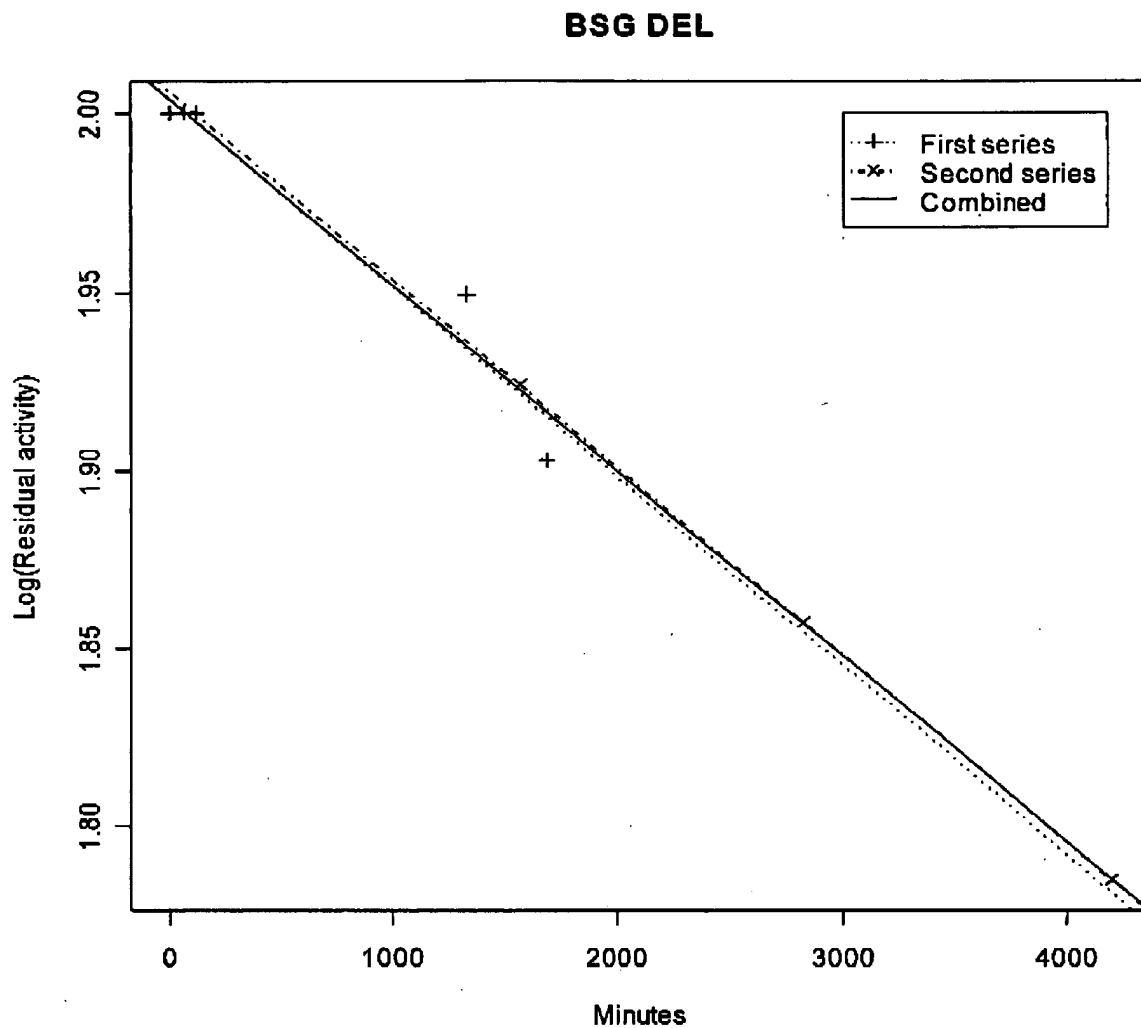
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.03408 on 20 degrees of freedom

Multiple R-Squared: 0.9768, Adjusted R-squared: 0.9733

F-statistic: 280.4 on 3 and 20 DF, p-value: < 2.2e-16

BSG-DEL

**Summary of statistical analysis.**

Below is a screen-dump from the statistical analysis, showing that there is not a significant difference in the slope in the two data series. The p-value for same slope is underlined.

The analysis was done in R version 1.8.1 (<http://www.r-project.org>). The data for the BSG deletion is held in the data-frame `bsg.del` as shown in the table above. In the data-frame the time is called `var1`, the residual activity

is var2 and var3 is a factor over the two series of experiments. The output shows the effect of the factor on a linear regression on the Log(residual activity) over incubation time.

```
> summary(lm(log10(bsg.del$var2)~bsg.del$var1*bsg.del$var3))
```

Call:

```
lm(formula = log10(bsg.del$var2) ~ bsg.del$var1 * bsg.del$var3)
```

Residuals:

```
      1      2      3      4      5      6      7      8
-0.0043196 -0.0008682  0.0020522  0.0151601  0.0002194 -0.0120245 -0.0004197
0.0002003
```

Coefficients:

```
              Estimate Std. Error t value Pr(>|t|)
(Intercept)    2.004e+00  5.978e-03  335.303 4.75e-10 ***
bsg.del$var1   -5.310e-05  6.243e-06  -8.505  0.00105 **
bsg.del$var34    1.836e-03  1.739e-02   0.106  0.92103
bsg.del$var1:bsg.del$var34  4.731e-07  8.218e-06   0.058  0.95685
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Residual standard error: 0.009979 on 4 degrees of freedom

Multiple R-Squared: 0.9905, Adjusted R-squared: 0.9834

F-statistic: 139.2 on 3 and 4 DF, p-value: 0.0001682

Comparing thermo stabilization.

Analysis of significance of different stabilization:

We have the following slopes on the curves:

	Slope	Std err	Relative std err
BAN WT	-0.346932	0.042031	0.121152
BAN DEL	-0.031550	0.001153	0.036556
BSG WT	-0.003286	0.000128	0.039024
BSG DEL	-0.000052	0.000002	0.040217

We can compute the ratios of the slopes (which are the reciprocals of the ratios of the half-lives).

	Ratio	Std err	Relative Std err
BAN WT / BAN DEL	10.9962346	1.3915416	0.1265471
BSG WT / BSG DEL	63.04430515	3.53290004	0.05603837

This means we have a ratio between the slopes of

$$\frac{63.0}{11.0} = 5.73$$

with a relative standard error of

$$\sqrt{0.127^2 + 0.056^2} = 0.138$$

and a standard error of

$$5.73 * 0.138 = 0.79$$

So, if we use the golden rule of standard errors, that the true value is within +/- two standard errors of the estimated value, we have that the deletion has a stabilizing effect in BSG which is between 4 and 7 times what is seen in BAN.

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Issued Patents:

US 5,753,460 (amylase variants)
US 5,801,043 (amylase variants)
US 5,830,837 (amylase variants)
US 5,989,169 (amylase variants)
US 6,022,724 (amylase mutants)
US 6,093,562 (amylase variants)
US 6,143,708 (amylase mutants)
US 6,159,687 (method for generating recombined polynucleotides)
US 6,159,688 (method of producing polynucleotide variants)
US 6,165,718 (method for in vivo production of a mutant library in cells)
US 6,187,576 ((amylase variants)
US 6,204,232 (amylase mutants)
US 6,291,165 (shuffling of heterologous DNA sequences)
US 6,297,038 (amylase variants)
US 6,309,871 (alkaline amylases)
US 6,326,206 (in vivo recombination)
US 6,361,989 (amylases)
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US 6,518,042 (diversity generation)
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